

The Experimental Production of Sarcoma by the Use of Beryllium Oxide.

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Osteogenic sarcoma was produced experimentally by the repeated injection of beryllium oxide (BeO) directly into the right femur of rabbits. Young rabbits weighing 1.5 kg on the average were injected with 1 cc of 1.0 % suspension of beryllium oxide in physiologic saline solution. Ten rabbits were treated with a single injection of beryllium. Forty-three rabbits were twice a week for a total of divided into five groups. Of 30 rabbits which survived for more than 9 months Twenty-five rabbits (84%) developed osteogenic sarcoma, chondrosarcoma or presarcomatous changes.

INTRODUCTION

Various tumors have been produced experimentally by many researchers since YAMAGIWA first produced in 1915. However, most of them are tumors other than those of the bone. This is probably based on the fact that the study of bone tumors was accompanied by more difficulties than that of soft tissue tumors. In 1946 GARDNER had produced osteogenic sarcomas in 7 rabbits by intravenous injection of a suspension of powdered alloy of beryllium, zinc and arsenic. This experiment was done accompanying the experimental study on the occupational disease, berylliosis, incidence of which was increasing among the workers in the factories of durable copper alloy goods such as radiation tubes, fluorescent lamps and vacuum tubes in which Beryllium was used in large amounts. This study became the start of the experimental study of bone tumors. The above fact attracted researchers attention to the pathological changes and its transition to tumor produced when beryllium compounds were administered. In the present study osteogenic sarcoma was produced experimentally by the repeated injection of beryllium oxide (BeO) directly into the bone marrow of rabbits. These induced sarcomas was studied histopathologically and histochemically.

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Table 1. Conditions and results of the experiment.

Reference number	Animal number	Number of BeO injection	Total experimental period	Period from time of final injection to first notice of tumors	Period from time of first notice of tumors to death	Natural death or slaughter	classification of induced tumors	Size of tumors (cm)
1	43	1	5 hrs			N.D.		
2	46	1	10 "			S.		
3	41	1	24 "			S.		
4	47	1	48 "			S.		
5	61	1	4 days	4 days		S.	Presarcomatoid change	No gross change
6	58	1	7 "			S.		
7	55	1	14 "			S.		
8	155	1	21 "			S.		
9	62	1	28 "			S.		
10	63	1	35 "	35 "		S.	Chondroma	No gross change
11	48	3	56 "			N.D.		
12	25	9	77 "			N.D.		
13	53	3	160 "			S.		
14	45	7	217 "			S.		
15	42	11	111 "			N.D.		
16	19	17	117 "			N.D.		
17	34	18	134 "			N.D.		
18	66	17	138 "			S.		
19	9	19	165 "			N.D.		
20	40	12	170 "			N.D.		
21	29	11	232 "			N.D.		
22	70	13	270 "	60 "	150 days	S.	Osteogenic sarcoma	2×1×1
23	44	13	307 "	60 "	187 "	S.	Presarcomatoid change	2×2×1
24	60	13	327 "			N.D.		
25	20	11	360 "	150 "	150 "	S.	Osteochondrosarcoma	7×6×5
26	26	19	360 "	13 "	60 "	S.	Osteogenic sarcoma	8×6×6
27	38	19	380 "	13 "	30 "	N.D.	Osteogenic sarcoma	4×4×3

28	56	19	380 "	20 "	30 "	N.D.	Osteogenic sarcoma	4×3×2
29	31	17	407 "	60 "	96 "	S	Osteogenic sarcoma	8×7×6
30	57	11	447 "	271 "	73 "	S.	Osteogenic sarcoma	6×6×4
31	8	26	184 "			N.D.		
32	68	22	196 "			N.D.		
33	27	24	327 "	20 "	30 "	S.	Osteochondrosarcoma	5×5×4
34	30	22	330 "	68 "	57 "	S.	Osteochondrosarcoma	9×6×4
35	65	22	334 "	192 "	42 "	S.	Chondrosarcoma	7×6×4
36	33	25	340 "	33 "	67 "	S.	Osteochondrosarcoma	8×7×6
37	35	24	352 "	22 "	90 "	S.	Osteogenic sarcoma	10×7×7
38	16	27	360 "	unknown	unknown	N.D.	Osteochondrosarcoma	No gross change
39	37	26	385 "	85 days	90 days	S.	Osteogenic sarcoma	4×3×3
40	32	25	433 "	133 "	60 "	S.	Osteogenic sarcoma	7×6×5
41	36	30	463 "	160 "	43 "	S.	Chondrosarcoma	10×9×7
42	39	21	534 "	151 "	21 "	N.D.	Osteogenic sarcoma	3×2×2
43	50	22	638 "	259 "	81 "	N.D.	Osteogenic sarcoma	5×5×3
44	14	31	249 "			N.D.		
45	22	38	250 "			S.		
46	15	39	285 "			N.D.		
47	17	40	293 "			N.D.		
48	18	38	296 "			N.D.		
49	13	39	340 "	21 "	30 "	S.	Osteochondrosarcoma	3×2×1
50	10	32	380 "	80 "	30 "	N.D.	Osteogenic sarcoma	4×3×3
51	67	31	394 "	205 "	39 "	S.	Osteogenic sarcoma	5×3×3
52	11	42	323 "	unknown	unknown	N.D.	Osteogenic sarcoma	No gross change
53	12	41	324 "	"	"	N.D.	Osteochondrosarcoma	No gross change
54	4	42	386 "			N.D.		
55	3	43	578 "	254 days	150 days	S.	Osteogenic sarcoma	11×10×8

MATERIALS AND METHODS

Young rabbits weighing 1.5 kg on the average were injected with 1 cc of 1.0 % suspension of beryllium oxide in physiologic saline solution into the metaphysis marrow of the right femur. As for the injector, a modified bone marrow aspirator was used. Ten rabbits were treated with a single injection of beryllium. Forty-five rabbits were given beryllium twice a week and divided into five groups: 2-10 injections, 11-20, 21-30, 31-40, and over 41. The induced tumors were cut into two, making two groups. No decalcification was done on one of the groups and this group was fixed in carnoy solution. The other group was fixed in 10 % formalin as control. The other organs involved by metastasis were also examined microscopically. The sections were stained with H. E., Azan staining (MALORY's method), VAN GIESON, PAS, Methyl green Pyronine, FEULGEN reaction, SCHNEIDER' T. C. A., ERICKSON, SAX et OGUR' P. C. A. etc. The survival duration of the rabbits, frequency of injection and period from the initial injection to the production of tumors are shown in Table 1.

RESULTS

I. Group treated with single injection:

The animal # 61 was slaughtered 4 days after single injection. In this case, an irregular proliferation of immature mesenchymal cells was seen around the injected Beryllium Oxide (hereafter abbreviated as BeO). This should be interpreted as presarcomatous change (Fig. 1). In other cases no evident findings consistent with tumor were seen. The experimental periods of these cases were much less than the total experimental period of 9 months (the period of sudden rise of occurrence rate of sarcomas) (Table 2).

II. Group treated with 2-10 injections:

Inflammatory signs or pictures of new-formation of the osteal tissue were seen in all cases but no evidence of tumor was noticed (Table 2).

III. Group treated with 11-20 injections:

The animal # 44 was treated with 13 injections. In this case the area of injection was grossly white and increased in consistency (Fig. 2), and histologically, chronic proliferation of bony trabeculae associated with irregular proliferation of rather hypochromic, spindle-shaped, roughly round, young cells with occasional patches of osteoid stroma, i. e., the early picture of osteogenic sarcoma was seen (Fig. 3). The animal # 26 which was treated with 19 injections developed a fracture of the femur 30 days before the final injection. After the final injection it developed an induration about 3 cm. in diameter and began to limp. After that the tumor increased in size to about 8×8 cm. (Fig. 4&5). This was confirmed as osteogenic sarcoma histologically. In the animal # 38 slight

Table 2.
Reactions of the bone in cases of BeO injection
(Figures represent animal number)

Number of BeO injection	1	2 - 10	11 - 20	21 - 30	31 - 40	41 -
Reaction not noticeable	43					
Degenerative change (Non-tumorous reaction)					17, 22	4
Reaction with noticeable inflammation (Non-tumorous reaction)	41, 46 55, 62 155	25 53	40 60			
Reaction composed mainly of progressive lesion. (Non-tumorous reaction)	47, 58	45 48	19, 34 42, 66	8, 68	14, 15	
Chondroma (Tumorous reaction)	63					
Osteoma (Tumorous reaction)			9, 29		18	
Presarcomatous change (Tumorous reaction)	61		44			
Osteogenic sarcoma (Tumorous reaction)			26, 31 38, 56 57, 70	32, 35 37, 39 50	10, 67	3, 11
Chondrosarcoma (Tumorous reaction)				36, 65		
Osteochondrosarcoma (Tumorous reaction)			20	16, 27 30, 33	13	12

swelling with induration was noticed in the injected area of the femur, and a cartilage-firm tumor measuring approximately $5 \times 4 \times 4$ cm. was seen in the head of the femur, far from the injected area (Fig. 6). This was disclosed as osteogenic sarcoma histologically. In the animal # 56 an induration about 3 cm. in diameter was noticed 20 days after the 19th injection, and the animal died 30 days after the final injection. Histologically, this was found to be an osteogenic sarcoma with less tendency to osteogenesis (Fig. 7). The animals # 20, # 31 and # 70 also showed typical pictures of osteogenic sarcoma or osteochondrosarcoma (Fig. 8).

IV. Group treated with 21-30 injections :

Of 13 cases, tumors were seen in all cases except for 2 cases of early death (100% in case the total experimental period is defined as 9 months). In the animal # 27 an induration approximately 3 cm. in diameter was noticed 20 days after the 24th injection. X-ray of the thigh showed osteogenic tumors approximately $5 \times 4 \times 4$ cm. in the knee area and at the upper end of the femur, far from the injected area (Fig. 9). These were osteochondrosarcoma histologically, and metastatic

lesions were seen in the lungs. In the animal # 30 a calcified lesion approximately 3 cm. in diameter was seen at the end of the femur, particularly in the flexor aspect, 90 days after the 22nd injection (Fig. 10). The tumor gradually increased in size after that. A month later, the toes became ulcerated and began to bleed followed by paralysis of the legs. When slaughtered the tumor was about $9 \times 6 \times 4$ cm. and bone-like in consistency, and there were many soy-bean sized, firm, white metastatic lesions in the lungs. The primary lesion was revealed as osteochondrosarcoma but the metastatic lesions in the lungs and liver were composed of loose connective tissue with irregular intercellular spaces filled with mucoid fluid, presenting an appearance resembling that of myxosarcoma, but chondroblasts were occasionally interlaced (Fig. 11). In the animal # 65 a tumor of the bone was found 192 days after the 22nd injection. The tumor increased in size to about $7 \times 6 \times 4$ cm. 234 days after the final injection, and at the same time a spontaneous fracture of the bone was noticed. Autopsy revealed a cartilage-firm tumor with rather soft, white cut surfaces, and histologic examination disclosed it to be a chondrosarcoma consisted mostly of markedly polymorphic young chondrocytes (Fig. 12). The metastatic lesions of the lungs, kidneys and lymph nodes of the lumbar region presented the same findings. In the animal # 35 a firm tumor approximately 3 cm. in diameter was noticed 22 days after the 24th injection. After that the tumor increased in size to approximately $10 \times 10 \times 8$ cm. involving the entire femur (Fig. 13). It was histologically an osteogenic sarcoma. The animal # 32 developed a spontaneous fracture and began to limp 73 days after the 25th injection. On the X-ray film taken 193 days after the final injection the tumor had involved the entire femur in association with marked osteogenesis (Fig. 14). Histologically, the tumor was an osteogenic sarcoma consisted of polymorphic cells, and numerous metastatic lesions varying in diameter from 0.5 cm. to 1.0 cm. were seen in the lungs (Fig. 15). The animals # 16, # 33, # 36, # 37, # 39 and # 50 also showed osteogenic sarcoma, chondrosarcoma or chondroosteosarcoma (Table 1 & 2).

V. Group treated with 31–40 injections :

Evidence of malignant tumors was noticed in 3 out of 6 cases (# 10, # 13 and # 67) (Table 1).

VI. Group treated with more than 40 injections :

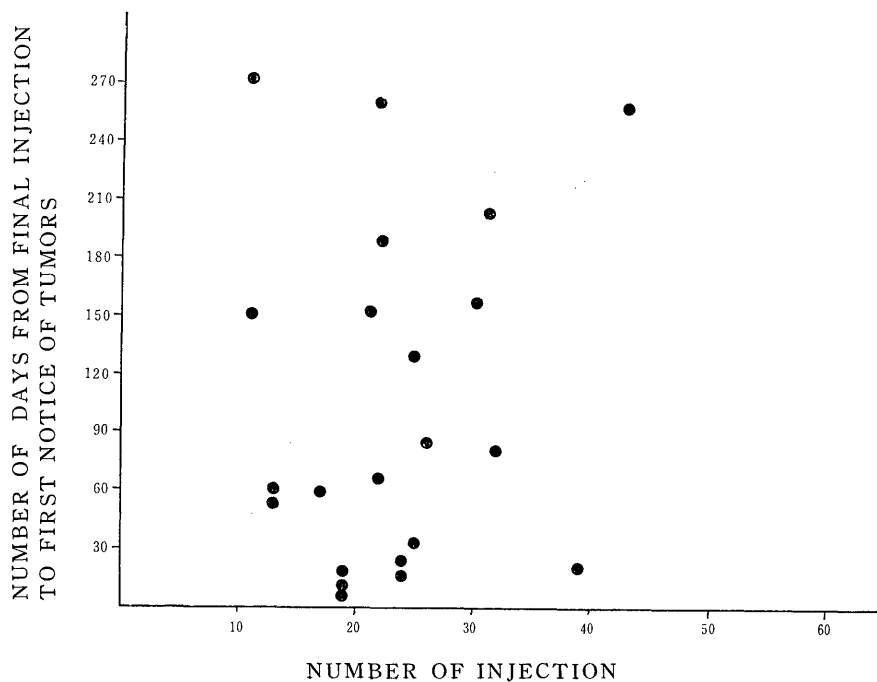
Slight swelling was noticed in the injected area in the animals # 11 and # 12. Malignant transformation was noticed only histologically in the area of the bone marrow. In the animal # 3 a tumor was noticed 254 days after the 43rd injection. After that the tumor gradually increased in size involving the entire femur (Fig. 16). Histologically, this was an osteogenic sarcoma with rather marked new-formation of bony trabeculae (Fig. 17). Metastatic lesions were seen in the lungs and liver, and these lesions all showed active osteogenesis (Fig. 18).

DISCUSSION

1) Summary of early changes caused by injection of BeO :

Under the author's experimental conditions, BeO was seen throughout the bone marrow 5 hours after the initial injection but no tissue reaction was noticed. However, more than 10 hours after the injection an increase of granulocytic young cells or young megakaryocytes was seen in many cases in addition to leukocytic reaction. Moreover, focal proliferation of young mesenchymal cells which seemed to have been derived from reticulocytic cells was shown. Particularly in the animal slaughtered 4 days after the injection, marked proliferation of spindle-shaped mesodermic cells which presented a picture similar to that of sarcoma was seen around the injected BeO. In the animal # 61 abnormal proliferation of osteoblastic cells accompanied by frequent karyokinesis, and in the animal # 44 marked proliferation of bony trabeculae was seen in addition to that. These pictures were all interpreted as presarcoma or early stage of sarcoma. These are considered to be the results of biological transformations caused by chemical or physical specific action of BeO on the mesodermic tissue. It is considered that if true sarcoma develop from these abnormal tissues, these osteoblasts might get mutation during the procedure of their proliferation and be led to

Fig. 19. Relationship between the number of injections and the period from the final injection to the first notice of tumors.

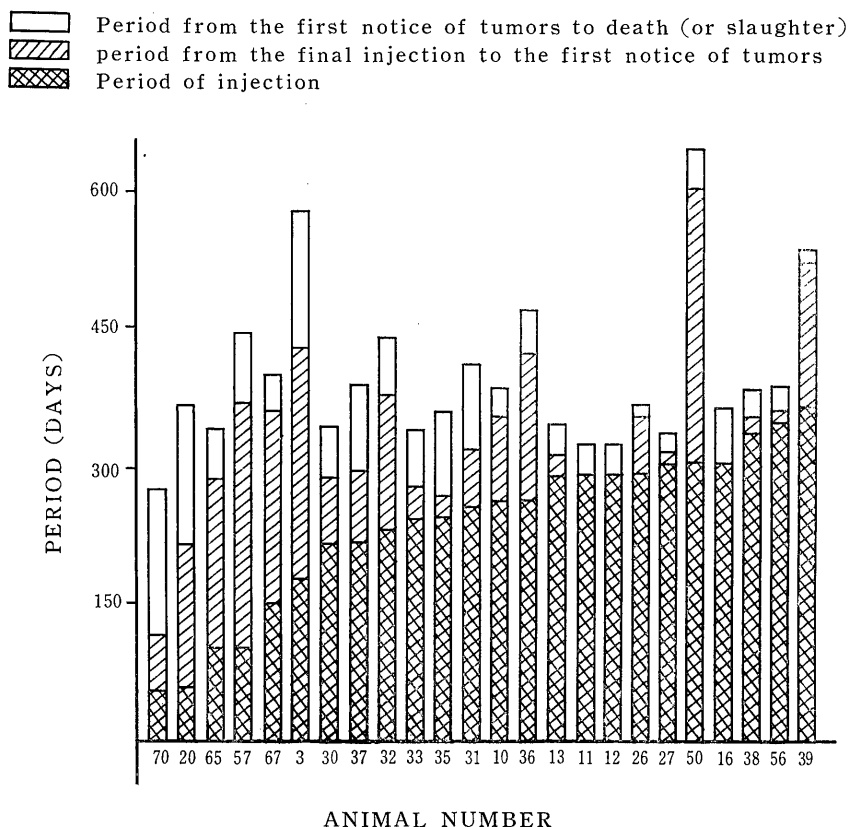


malignant transformation, acquiring unusual energy.

2) Periods of the production and the production rate of malignant tumors :

In the author's experiments, tumors transformation was noticed in 8 out of 9 animals of the group treated with 11–20 injections, which survived for more than 9 months. The remaining one died accidentally. The picture of sarcoma was seen histologically in 7 out of these 8 animals. Tumors were produced in all 11 animals of the group treated with 21–30 injections, which survived for more than 9 months. As shown in Table 2, the production of tumors is concentrated to this group. The average period from the final injection to the first notice of tumors was 12 weeks. But there is no distinct relationship between these periods and the number of injections (Fig. 19). In the experiments by KELLY et al. the production of tumors was noticed after 30 to 52 weeks after the final injection in the group treated with a total of 20 intra-venous injections, 2 injections a week. However, in the author's experiments, tumors were produced in less than half the period after the final

Fig. 20. Relationship between the first notice of tumors and the periods from the initial injection and from the final injection to the first notice of tumors.



injection. The durations of injection, periods from the final injection to the first notice of tumors and total experiment-periods in cases having macroscopic tumors are showed in Fig. 20. Tumors were produced in a rather small number of animals among those treated with more than 30 injections: 3 out of 6 animals of the group treated with 31–40 injections and 3 out of 4 animals of the group treated with more than 40 injections. On the basis of the fact that the groups treated with more than 30 injections required the same or a longer period from the final injection to the production of tumors in comparison with the group treated with 21–30 injections, the treatment with 20–30 injections is the best method which the author would like to recommend.

3) Metastases of sarcoma:

In the author's experiments, metastases were seen in 15 out of 24 animals with sarcoma, about 62 %. As shown in Table 3, metastasis to the lung was seen in all 15 cases. Other than that, metastasis were seen also in the liver, kidneys and lymph nodes of the lumbar region. In the most severe case, as shown in Fig. 15 the majority of the lung

Table 3.
Metastasis of the induced tumors.

Animal number	Lung.	Liver	Kidney	Spleen	L. N. s of lumbar region	Histologic pictures of metastatic lesions.
3	+	+	—	—	—	Osteogenic sarcoma
10	—	—	—	—	—	
11	—	—	—	—	—	
12	—	—	—	—	—	
13	—	—	—	—	—	
16	—	—	—	—	—	Chondrosarcoma
20	+	—	—	—	—	
26	+	—	—	—	—	
27	+	—	—	—	—	Osteogenic sarcoma
30	++	+	—	—	—	Myxosarcoma
31	+	—	—	—	—	Osteogenic sarcoma
32	+++	—	—	—	—	Osteogenic sarcoma
33	+	+	—	—	—	Myxosarcoma
35	+	—	—	—	+	Osteogenic sarcoma
36	+	—	—	—	—	Osteogenic sarcoma
37	+	—	—	—	—	Osteoid sarcoma
38	++	—	—	—	—	Osteogenic sarcoma
39	—	—	—	—	—	Osteogenic sarcoma
50	—	—	—	—	—	
56	++	—	—	—	—	
57	—	—	—	—	—	Chondrosarcoma
65	+++	+	+	—	+	
67	+	—	—	—	—	
70	—	—	—	—	+	Osteogenic sarcoma

parenchyma was occupied by metastatic lesions. Histologically, the metastatic lesion were composed of young tissues in comparison with the primary lesions. But the metastatic lesions in the lymph nodes were particularly firm and showed osteal tissue in large amounts.

SUMMARY

1) By experimental BeO-injection in the bone marrow, out of 30 rabbits which survived for more than 9 months 25 cases (84%) developed osteogenic sarcoma, chondrosarcoma or presarcomatous changes.

2) Metastatic lesions were noted mostly in the lung, but occasionally also in the liver, kidneys and lymph nodes. The frequency of metastasis was found to be 68%.

3) The number of rabbits developing tumors were as follows:

11–20 injections: 8 of 9 (87%), 21–30 injections: 11 of 11 (100%), 31–40 injections: 3 of 6 (50%), and over 41 injections: 3 of 4 (75%). Two injections of beryllium per week for a total of 21–30 doses appears to be the most effective schedule for the production of sarcomas. It should be noteworthy that there were some cases in which only microscopic examination could detect the sarcoma although no sign of tumor was noticed macroscopically.

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EXPLANATION OF FIGURES

- Fig. 1. Histologic picture of the femur of animal #61 (4 days after the initial injection). Immature mesodermic cells are irregularly arranged around the homogenous mass of BeO on the right. H. E.
- Fig. 2. #44 (treated with 13 injections. 247 days after the final injection). A bone-firm area is seen in the lower one third. (arrowed)
- Fig. 3. Histologic picture of the same case. Spindle-shaped, roughly round, young cells are irregularly proliferating accompanied by small bony trabeculae. H. E.
- Fig. 4. #26 (treated with 19 injections). X-ray picture taken 350 days after the initial injection. A tumor mottled with areas of various degrees of density associated with a fracture is seen in the lower half of the thigh.
- Fig. 5. Photograph of the sarcoma of the same case.
- Fig. 6. X-ray picture of animal #38 (died 43 days after the 19th injection) taken 375 days after the initial injection. Swelling of the lower end of the thigh and a hen's egg-sized, light, tumorous shadow near the head of the femur.
- Fig. 7. #56 (died 50 days after the 19th injection). Histologic picture of the tumor of the thigh. Sarcomatoid proliferation of spindle-shaped cells. New-formation of osteoid tissue is seen in the upper portion. H. E.
- Fig. 8. #31 (treated with 17 injections). X-ray picture taken 156 days after the final injection. A large tumor involving the entire length of the femur.
- Fig. 9. #27 (slaughtered 50 days after the 24th injection). X-ray picture taken 326 days after the initial injection. Osteal tumors are seen near the injected area (upper portion) and at the head (lower portion).
- Fig. 10. X-ray picture of #30 taken 90 days after the final (22nd) injection. A tumor of increased density is seen with the lower end of the femur as the center.
- Fig. 11. The same case. Metastatic lesions in the liver. Irregular-shaped (spindle-shaped or round) cells are coarsely proliferating, presenting a picture which resembles that of myxoma. Chondroblast-like cells also are interlaced. H. E.
- Fig. 12. Tumor of the thigh of #65 (slaughtered 234 days after the 22nd injection). Composed of markedly polymorphic young cartilagenous cells. H. E.
- Fig. 13. Tumor of the thigh of #35 (112 days after the 24th injection).

- Fig. 14. #32. (treated with 25 injections). X-ray picture taken 193 days after the final injection. Tumorous transformation is marked throughout the thigh.
- Fig. 15. Metastatic lesions of the lungs of the same case.
- Fig. 16. #3 (404 days after the 43rd injection). Cut surface of the tumor of the thigh.
- Fig. 17. Histologic picture of the same case. Rather large bony trabeculae and irregular proliferation of osteoblasts are seen.
- Fig. 18. Metastatic lesions of the lung of the same case. Peripheral sarcomatous cells are very young but the bony trabeculae of the central region (lower portion) are rather highly differentiated.

